Prevalence, drug resistance spectrum and virulence gene analysis of *Campylobacter jejuni* in broiler farms in central Shanxi, China

Huilin Yang, Yongbin Li, Yu Zhang, Bowen Dong, Buting Duan, Lulu Guo, Tingyang Wang, Xiaoling Lv, Mingxue Zheng, Xiaozhen Cui, and Rui Bai[1]

College of Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi, 030801, China

ABSTRACT This study collected 324 chicken cloacal swabs from 6 broiler farms in 4 different areas in Shanxi Province, China (i.e., Lvliang, Taiyuan, Jinzhong, and Yangquan), and analyzed the antimicrobial resistance and virulence-associated genes of the isolates to investigate the prevalence, drug resistance, and virulence gene data of *Campylobacter jejuni* in broilers. The population structure of *C. jejuni* and genetic evolutionary relationships among isolates from broiler farms in different regions were studied by using multilocus sequence typing. A total of 35 *C. jejuni* isolates with an infection rate of 10.8% (35/324) were obtained. The isolates were most resistant to ampicillin (85.7%) and were most sensitive to erythromycin (14.3%). Isolates with multidrug resistance accounted for 88.6% of the total isolates. In this experiment, 15 distinct sequence types were identified and included 9 new unique sequence types. *cad*F was present in all isolates, and *cia*B had the lowest prevalence (51.4%). *C. jejuni* collected from broiler farms in central Shanxi had varied infection rates, and their overall positive rate was lower than of *C. jejuni* collected from other regions of the country. The isolates had high resistance to quinolones and β-lactams, and multidrug resistance was prevalent. The isolates were genotypically diverse and carried 5 virulence-associated genes at high rates. Therefore, the importance of source contamination control in broiler farms is emphasized and may have considerable effects on human and animal health.

Key words: *Campylobacter jejuni*, isolation and identification, drug resistance, MLST typing, virulence genes

2023 Poultry Science 102:102419
https://doi.org/10.1016/j.psj.2022.102419

INTRODUCTION

Campylobacter is a zoonotic pathogen that is spread through food. *Campylobacter jejuni* is the most common pathogenic member of this genus and is the primary cause of bacterial gastroenteritis in people worldwide (Man, 2011). Fever, diarrhea, stomach pain, nausea, and vomiting are the most common clinical symptoms of campylobacteriosis. The World Health Organization (**WHO**) estimates that *Campylobacter* causes more than 37,000 deaths each year worldwide (Goualie et al., 2019).

Poultry is widely recognized as the natural host of *Campylobacter* species. *Campylobacter* colonization rates in broiler flocks range from 3% to 90%, with the highest rates found in flocks older than 3 wk (Marotta et al., 2015; Tangkham et al., 2016). The misuse of antibiotics has led to the emergence of selectively resistant *C. jejuni* in chickens in poultry farms (Cerf et al., 2010). Antimicrobial resistance in animals has serious implications for humans primarily because poultry is one of the animal vectors that can expose humans to infection and their contamination with resistant strains can lead to the spread of resistance through the food chain (EFSA Panel on Biological Hazards, 2009).

Comparing and determining the source of infection and route of transmission is difficult due to the high molecular diversity of *C. jejuni* (Kaakoush et al., 2015). Multilocus sequence typing (**MLST**) is a genotyping approach that is frequently used for the molecular typing of *C. jejuni* isolates because of its high resolution (Dingle et al., 2001) and may be applied to trace the genetic origin of pathogenic bacteria. MLST uses the genetic variation of 7 housekeeping loci of *C. jejuni* to determine molecular relationships among isolates. An open database of *Campylobacter* is available at PubMLST (http://pubmlst.org/campylobacter).

The cecum is the main colonization site of *C. jejuni* in poultry. Intestinal damage is accompanied by motility, adhesion, invasion, and toxin production after colonization (Epps et al., 2013). Some genes, such as flagellin

Received October 28, 2022.
Accepted December 5, 2022.

[1]Corresponding author: chinabairui@sxau.edu.cn

*fla*A, fibronectin *cad*F, and cytokinin *cdt*ABC, have been identified to be associated with the pathogenicity of *C. jejuni* (Nguyen et al., 2016).

Humans currently consume nearly 60 billion chickens per year, more than any other food of animal origin (Colles et al., 2016). Reports and epidemiological data on *C. jejuni* in Shanxi Province, China, are scarce. Hence, the purpose of this study is to investigate the prevalence, drug resistance spectrum, and virulence genes of *C. jejuni* in broiler farms, which are the main pollution source in some regions of Shanxi Province, China and to analyze the genetic diversity of *C. jejuni* by using MLST typing.

MATERIALS AND METHODS

Ethical Statement

No animal experiments were carried out in the course of this study. And the cloacal swabs of chickens collected from each farm did not compromise animal welfare. The whole process did not cause panic among the chickens, nor did it affect the physical condition and subsequent life of the collected individuals.

Sample Collection and Preparation

Six broiler farms in 4 central regions, all of which provide live chickens or chicken meat to local markets, were sampled from July to October 2021. The desired sample size was derived by using Thrusfield's formula (Thrusfield, 2018) on the basis of a 95% confidence interval and a sample size with 5% precision for the estimated prevalence of *C. jejuni*. The broilers in the slaughterhouse were 8-wk-old white-feathered broilers. A total of 324 samples of chicken cloacal swabs were obtained throughout the experiment. The sample size collected varied depending on the size of each broiler farm as follows: Lvliang Farm I (n = 54), Taiyuan Farm II (n = 45), Jinzhong Farm III (n = 116), IV (n = 37), Yangquan Farm V (n = 46), and VI (n = 26).

Establishment of an Identification Method for C. Jejuni

The 16SrRNA gene of *Campylobacter* spp and the specific *hipO* gene (Z36940.1) of *C. jejuni* were selected to design primers to establish a double PCR identification method. The primer sequences are shown in Table 1. A MightyAmp Genotyping Kit (TAKARA, Kyoto, Japan) was used to perform PCR amplification with a total volume of 25 μL, which contained 12.5 μL of 2 Mighty Amp Buffer (4 mM Mg^{2+}), 0.5 μL of Mighty Amp DNA Polymerase (1.25 U/L), 1.25 μL of chromosomal DNA template, 1.25 μL of the upstream and downstream of each of the 2 primer pairs, and 5.75 μL of sterile distilled water. The reaction conditions for 30 cycles were incubation at 98°C for 2 s, denaturation at 98°C for 10 s, annealing at 56.4°C for 15 s, and extension at 68°C for 45 s. PCR products were electrophoresed on 1.2% agarose gel and photographed by using a WD-9413B gel imaging analysis system (LIUYI, Beijing, China). The suspected samples were confirmed to be positive through comparison with the standard bands of *C. jejuni* (ATCC33560).

Isolation and Identification of C. Jejuni

After cloacal swab samples were collected from broiler farms with sterile swabs, the swabs were deposited in 10 mL centrifuge tubes containing Cary−Blair transport medium (Hopebiol, Qingdao, China) and transported on ice to the laboratory within 6 h. The samples were tested promptly after their arrival. Each swab was placed in a test tube containing 5 mL of Bolton broth (Hopebiol), then placed in an anaerobic culture tank (MITSUBISHI MGC ANAEROBIC CULTURE, Tokyo, Japan) under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) provided by a microaerobic gas-producing bag (Hopebiol), and incubated at 42°C for 48 h. Each sample was dipped into the enriched broth by using a sterile inoculation loop and scribed in 3 zones on Skirrow blood agar plates (modified Skirrow agar). Then, they were placed in an anaerobic culture jar with a microaerobic environment and incubated for 48 h at 42°C.

Suspect colonies were chosen from the Skirrow plates for DNA extraction and double PCR identification. First, DNA was extracted by using a crude method, and suspicious single colonies were selected, suspended in centrifuge tubes holding 120 μL of sterile water, boiled in water for 10 min, and centrifuged at 34,613 × *g* for 5 min. The supernatant obtained was DNA, which was used as the PCR template. The established PCR method was then used for identification.

Antimicrobial Susceptibility

The disk diffusion technique suggested by the WHO was utilized to detect *C. jejuni* drug resistance in accordance with the Institute of Clinical and Laboratory Standards (**CLSI**) guidelines (Fothergill, 2012). The sensitivity to the following drug tablets was tested:

Table 1. Primers used for duplex PCR assays.

Organism	Primer	Sequence (5′-3′)	Amplicon length (bp)
Campylobacter spp.	*16SrRNA*	F:ATCTAATGGCTTAACCATTAAAC R:GGACGGTAACTAGTTTAGTATT	857 (Zendehbad et al., 2013)
C. jejuni	*hip*O	F:GTAAAGCCACAATAAGCAA R:AAGGCAATGATAGAAGATG	229

gentamicin (10 μg), ampicillin (10 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), tetracycline (30 μg), erythromycin (15 μg), nalidixic acid (30 μg), clindamycin (2 μg), and cefotaxime (30 μg). All antibacterial sensitivity tests were performed on Mueller −Hinton (**MH**) agar plates. The concentration of the bacterial suspension was adjusted to 0.5 McFarland (approximately 1×10^8 CFU/mL). Then, 120 μL of the suspension was evenly spread onto MH agar plates with a disposable sterile applicator stick and incubated for 10 min at 42℃. The drug-sensitive paper was then adhered to the surface of agar by using sterile tweezers and cultivated for 24 h under microaerobic conditions at 42°C. The size of the bacteriostatic ring was observed. The bacteriostatic ring's edge was restricted to the visible development of bacteria that were not apparent to the naked eye. Qualified plates were taken out for measurement, and plates without bacteriostatic ring formation or with obvious bacterial undergrowth were further cultured in the culture tank, then taken out and measured after 48 h. The diameter range (mm) of each drug tablet's inhibition ring was measured independently. In accordance with the CLSI judgment criteria, the isolates were classified as sensitive, intermediate, or resistant on the basis of the size of the inhibition ring in the culture medium. The judgment criteria are detailed in Table 2. An isolate was considered multiresistant if it was resistant to 3 or more unrelated antimicrobial medicines (Maesaar et al., 2016).

MLST System

MLST was carried out as described by Colles and Maiden (2012) and Dingle et al. (2001). Seven housekeeping genes (*asp*A, *gln*A, *glt*A, *gly*A, *pgm, tkt*, and *unc*A) were included in the scheme. The primers for gene amplification and sequencing are available on the *Campylobacter* MLST website (http://pubmlst.org/campylobacter). The reaction conditions were designed in accordance with the instructions for the GoTaq Green Master Mix (Promega, Madison, WI) kit and the sequence length. The settings were as follows: 2 min of incubation at 95℃, 30 s of incubation at 95℃, 1 min of incubation at 50℃, 1 min of incubation at 72℃, 30 cycles, and 5 min of incubation at 72℃. Each 25 μL reaction system included 12.5 μL of GoTaq Green Master Mix, 1 μL each of upstream and downstream primers (10 μM), 2 μL of chromosomal DNA template, and 8.5 μL of sterile distilled water. The purification and sequencing of PCR products were performed by BGI Genomics (Beijing, China). The sequenced sequences of different housekeeping genes of *C. jejuni* were submitted to the MLST database for comparison and analysis, and the sequence type of the strain was determined on the basis of the sequence number of the matching alleles. A clonal complex is a group of sequences consisting of similar sequence-type strains. The sequence was then sequenced and introduced into MEGA7 software to construct a relational branching diagram of the isolated *C. jejuni* strains. New alleles and STs were submitted to the PubMLST database.

Identification of Putative Campylobacter Virulence Genes

The presence of 6 *Campylobacter* virulence genes (*cad*F, *fla*A, *che*Y, *cdt*A, *cdt*B, and *cdt*C) was determined by using PCR. The primers used in this study and the optimized conditions are shown in Table 3.

Data Analysis

The chi-square test in IBM SPPS Statistics 26.0 (IBM Inc., Armonk, NY) was used to examine differences across categories within defined groups. Fisher's exact test in the chi-square test was used to examine the variability of separate positive rates. $P < 0.05$ was considered significant for the variability of broiler farm samples in each region. Histograms were prepared with GraphPad Prism 6.0 software (San Diego, CA).

RESULTS

Isolation and Prevalence of C. Jejuni From Chicken

Double PCR identification detected 35 (10.8%) positive samples of *C. jejuni* and 8 (2.5%) other strains of *Campylobacter* spp among the 324 collected cloacal swabs. The identification results of some strains are shown in Figure 1. Broiler farms in Yangquan had the lowest positive rate of 2.8%, and those in Lvliang and

Table 2. Antimicrobial name and judging criteria.

Category	Antibacterial agents	Dosage (μg)	Criterion (mm)		
			Resistance (R)	Intermediate (I)	Sensitivity (S)
Macrolides	Erythromycin	15	≤13	14-22	≥23
	Clindamycin	2	≤15	16-18	≥19
Quinolones	Norfloxacin	10	≤12	13-16	≥17
	Ciprofloxacin	5	≤15	16-20	≥21
	Nalidixic acid	30	≤13	14-18	≥19
	Levofloxacin	5	≤13	14-16	≥17
Aminoglycoside	Gentamicin	10	≤12	13-14	≥15
Admidia-lactam	Ampicillin	10	≤13	14-16	≥17
	Cefotaxime	30	≤22	23-25	≥26
Tetracyclines	Tetracycline	30	≤11	12-14	≥15

Table 3. PCR primers for virulence gene.

Primer	Sequence (5′→3′)	Size (bp)	Reference	T annealing
cad-F	TTGAAGGTAATTTAGATATG	400bp	(Konkel et al., 1999)	45℃
cad-R	CTAATACCTAAAGTTGAAAC			
fla-R	AATAAAAATGCTGATAAAACAGGTG	855bp	(Hickey et al., 2000)	53℃
fla-F	TACCGAACCAATGTCTGCTCTGATT			
cdt-F	CAGAAAGCAAATGGAGTGTT	620bp	(Datta et al., 2003)	55℃
cdt-R	AGCTAAAAGCGGTGGAGTAT			
cia-F	TTTTTATCAGTCCTTA	986bp		42℃
cia-R	TTTCGGTATCATTAGC			
dna-F	AAGGCTTTGGCTCATC	720bp		46℃
dna-R	CTTTTTGTTCATCGTT			
che-F	CCCTAACAAGACTTGGACACGAT	407bp		55℃
che-R	TACCGCTCTTCTACGATAAAG			

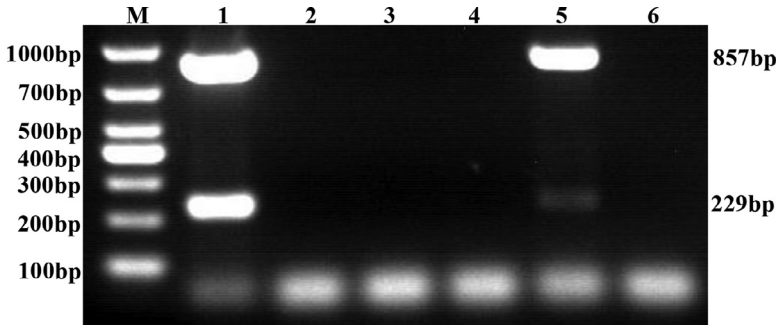

Figure 1. Detection samples results of duplex PCR. M: DNA Marker DL1000; 1−5: separate samples; 6: Negative control.

Table 4. Prevalence of *C. jejuni* in each poultry farm.

Region	No. of samples	No. (%) of positive samples
Lvliang	54	2 (3.7 ± 1.9)[b]
Taiyuan	45	3 (6.7 ± 3.8)[a,b]
Jinzhong	153	28 (18.0 ± 6.5)[a]
Yangquan	72	2 (2.8 ± 1.4)[b]

[a,b]Different lowercase letters in the table indicate statistical difference ($P < 0.05$).

Taiyuan had the positive rates of 3.7% and 6.7%, respectively. The highest positive rate of 18.0% was shown by broiler farms in the Jinzhong area and was significantly different from the positive rates of broiler farms in the Lvliang ($P < 0.05$) and Yangquan ($P < 0.05$) areas. No significant differences were found between broiler farms in Taiyuan and those in the other 3 areas. The details are shown in Table 4.

Antimicrobial Susceptibility Testing

All isolates were tested for antimicrobial resistance. The resistance rates of 10 antimicrobial drugs are illustrated in Figure 2. Multiple antimicrobial resistance profiles were determined (Table 5). The isolates had the highest rate of resistance to ampicillin (85.7%) then to quinolones (norfloxacin and nalidixic acid) and cefotaxime (both 80.0%).

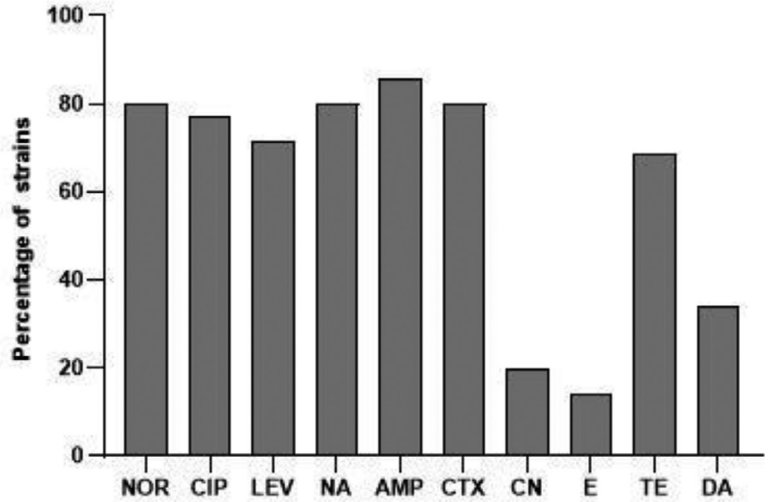

Figure 2. Antimicrobial resistance of *C. jejuni*. Abbreviations: AMP, Ampicillin; CIP, Ciprofloxacin; CN, Gentamicin; CTX, Cefotaxime; DA, Clindamycin; E, Erythromycin; LEV, Levofloxacin; NOR, Norfloxacin; NA, Nalidixic acid; TE, Tetracycline.

Table 5. Antimicrobial resistant profiles of *C. jejuni* isolates.

Antimicrobial resistance profile	No. of isolates	Percentage of isolates (%)
CN-LEV-DA-CTX	2	8.6
AMP-NOR-TE-DA-CTX	1	2.9
AMP-NOR-CIP-NA-CTX	2	5.7
CN-AMP-NOR-CIP-NA-DA	2	5.7
AMP-NOR-CIP-LEV-NA-CTX	3	8.6
CN-AMP-NOR-CIP-LEV-TE-NA	2	5.7
AMP-NOR-CIP-LEV-TE-NA-CTX	10	28.6
AMP-NOR-CIP-LEV-TE-NA-DA-CTX	4	11.4
CN-AMP-NOR-CIP-LEV-TE-NA-CTX	1	2.9
AMP-NOR-CIP-LEV-TE-E-NA-DA-CTX	3	8.6
CN-AMP-NOR-CIP-LEV-TE-E-NA-DA-CTX	1	2.9

Abbreviations: AMP, Ampicillin; CIP, Ciprofloxacin; CN, Gentamicin; CTX, Cefotaxime; DA, Clindamycin; E, Erythromycin; LEV, Levofloxacin; NA, Nalidixic acid; NOR, Norfloxacin; TE, Tetracycline.

In addition, the rate of resistance to ciprofloxacin, levofloxacin, and tetracycline was 67.0%, that to clindamycin and gentamicin was 14.3%, and that to erythromycin was only 5% and was the lowest. In this study, 12 antimicrobial resistance profiles were identified, with 32 strains (91.4%) being resistant to 2 or more antimicrobial agents. In *C. jejuni*, the AMP−NOR−CIP−LEV−TE−NA−CTX mode (28.6%) was the most common drug resistance mode, followed by the AMP−NOR−CIP−LEV−TE−NA−DA−CTX mode (11.4%), with the overall incidence of multiple drug resistance of 88.6%.

MLST Analysis

A total of 15 sequence types belonging to 6 clonal complexes and 7 individual plants were identified. *gly*A, *pgm*, and *unc*A showed the highest number of different allele values of 10 each, followed by *asp*A, *gln*A, and *glt*A with 8 each. *tkt* showed the lowest with 7. Eight new alleles were found. Nine new sequence types were discovered among the 35 isolates sequenced, 17 of which belonged to new sequence types and 18 were not allocated to clonal complexes and accounted for 51.4% of the total isolates. The most common sequence type isolated was 2274 (20.0%, 7/35). For clonal lines, the most abundant clonal complex was ST-354 (14.3%, 5/35). The sequence matching results of housekeeper gene amplification are shown in Table 6.

Eight strains of *C. jejuni* were selected from the MLST database of *C. jejuni*. These strains belonged to 7 sequence types, of which 4 were isolates from feces of patients with gastroenteritis, 2 (ID: 4552, 27649) were from China, and the other 2 strains (ID: 929, 11004) were from the United States and Korea. Among the 4 isolates of chicken origin, 2 (ID: 24438, 31042) were isolated from China and the other 2 strains (ID: 10598, 11493) were from Vietnam and Thailand.

In this experiment, clones of the same sequence type in the same large branch with close genetic distances included ST-354 (11351 and 11353), ST-21 (6684 and 6500), ST-353 (7452 and 1075), ST-828 (11350 and 6963), and ST-42 (469 and 9972). Two sequences of ST-42 (469 and 9972) were genetically farther apart than those of the other 4 clonal lines. 11127/ST-464 and 11317/ST-354 originated from broiler farms in Lvliang. 9049 and 2304 were from broiler farms in Yangquan. 6500/ST-21 and 2274 were collected from broiler farms in Taiyuan. The sequence types of the isolates from each region were in the same small branch, indicating the close genetic distances of the isolates originating from broiler farms in the same area. The broiler farms in the Jinzhong region contained all other typologies, except for 11312, indicating that *C. jejuni* isolates of chicken origin in the Jinzhong region were genetically diverse. The genetic distance of the isolates in this experiment was different from that of the isolates selected from the typing system. However, 5860/ST-460, which is of Vietnamese chicken origin, was in an independent branch and was distant from other genotypes. The other isolates had a certain genetic relationship with different isolates from central Shanxi, China. 3905/ST-45, 469/ST-42, and 5044/ST-257 from Chinese, American, and Korean human feces were genetically close to 9972/ST-42, 11127/ST-464, and 51/ST-443 from chicken sources, respectively. The details of the phylogenetic relationships of *C. jejuni* isolates are shown in Figure 3.

Table 6. Allele numbers, sequence types, and clonal complexes of *C. jejuni* isolates.[a]

			MLST profile						
Clonal complex	ST	No. of isolates	*asp*A	*gln*A	*glt*A	*gly*A	*pgm*	*tkt*	*unc*A
ST-21complex	6500	3	2	1	52	3	23	100	5
ST-42complex	9972	2	1	2	42	4	1025	9	467
ST-354complex	**11317**	1	8	10	2	2	956	863	**711**
	11351	2	8	10	2	2	10	12	**713**
	11353	2	8	10	89	2	10	12	**713**
ST-443complex	51	2	7	17	2	15	23	3	12
ST-464complex	**11127**	2	24	**786**	2	12	687	3	1
ST-828complex	**11350**	3	33	39	30	82	350	3	17
Singletons	2274	7	9	17	5	10	350	3	3
	2304	2	2	4	5	25	11	3	5
	9049	2	2	4	5	25	22	3	5
	11306	3	9	17	5	10	350	3	**710**
	11308	1	9	17	95	10	**1130**	762	3
	11312	1	2	1	52	**889**	23	100	5
	11318	2	37	403	29	64	**1132**	29	40

[a]Novel alleles and STs are in boldface.

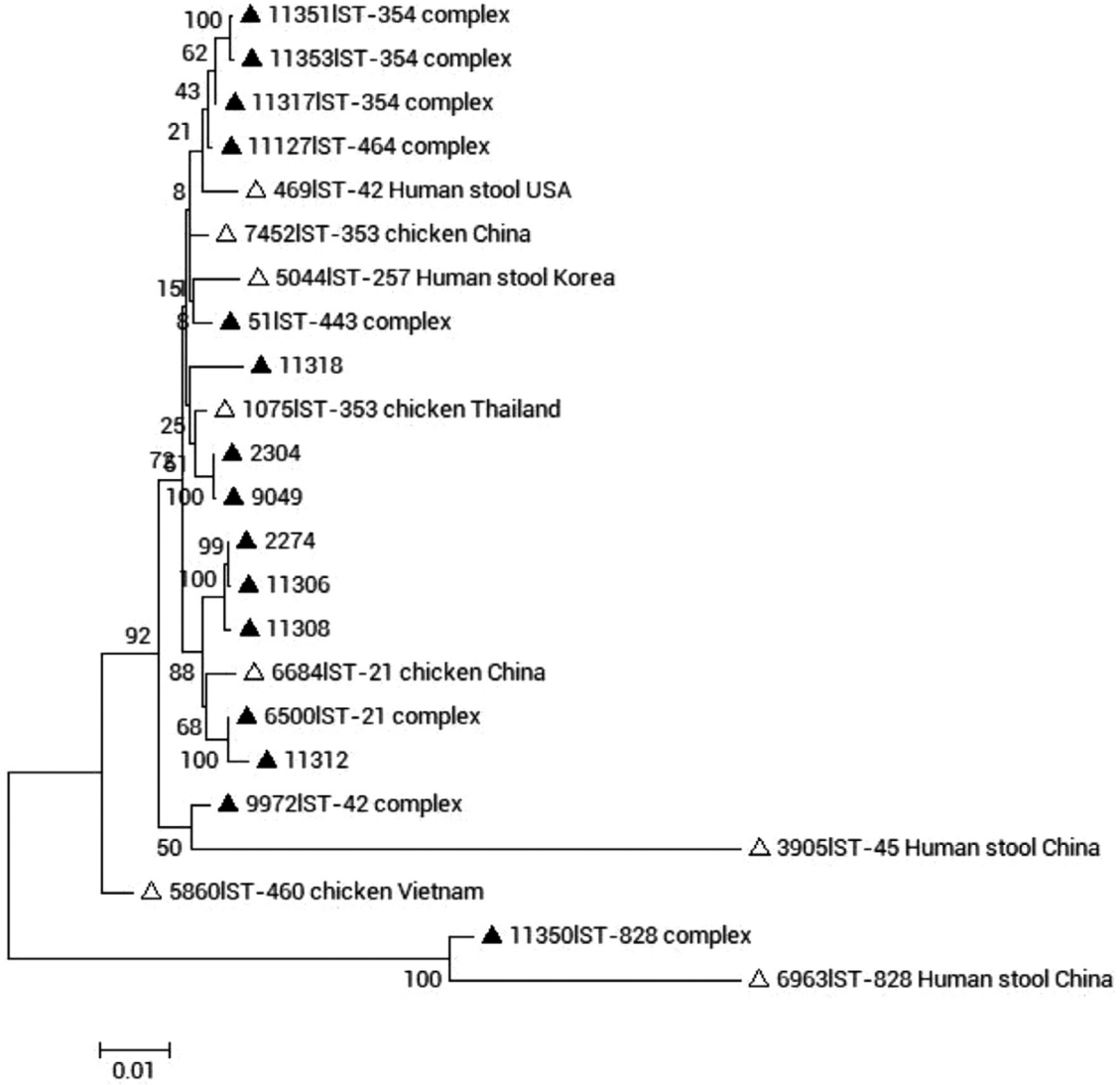

Figure 3. Cluster analysis diagram of sequence types of isolates. Note: The black triangle represents thesequence types of the strains isolated in this test are indicated and the white triangle represents the sequence types of isolates selected from the database.

Prevalence of Virulence Genes

All 6 virulence genes were amplified in the isolated *C. jejuni* strains with specific bands consistent with the expected target fragment size (Figure 4).

The overall detection rates of the 6 virulence-related genes in the 35 isolates of *C. jejuni* are shown in Table 7.

Among these genes, the virulence factors *cad*F and *fla*A associated with adhesion colonization had the detection rates of 100.0% and 94.3%, respectively; the chemotaxis-related virulence factor *che*Y had the detection rate of 94.3%; the toxin regulatory gene *cdt*B had the detection rate of 97.1%; the invasion-related gene *cia*B had the carriage rate of 51.4%; and heat shock-related protein

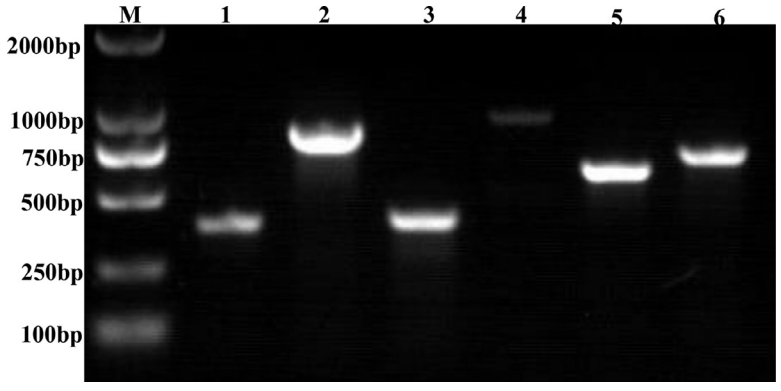

Figure 4. Results of amplification of 6 virulence genes in *C. jejuni*. M: DNA Marker DL2000; 1: *cad*F; 2: *fla*A; 3: *che*Y; 4: *cia*B; 5: *cdt*B; 6: *dna*J.

Table 7. Distribution of virulence gene in tested strains.

Virulence genes	No. of tested strains	Relevance ratio
*cad*F	35	35 (100.0%)
*fla*A		33 (94.3%)
*che*Y		33 (94.3%)
*cia*B		18 (51.4%)
*cdt*B		34 (97.1%)
*dna*J		34 (97.1%)

*dna*J had the carriage rate of 97.1%. Except for that of *cia*B, which had a low carriage rate, the detection rate of all 5 virulence genes exceeded 94.0%. Eighteen of the 35 *C. jejuni* isolates tested had all 6 virulence genes; 14 had 5 virulence genes; only 1 had 4 virulence genes; and 2 had 3 virulence factors.

DISCUSSION

Campylobacter contamination in the chicken production chain and retail meat varies greatly between nations, necessitating the development of country-specific intervention strategies (Wangroongsarb et al., 2021). According to a collection of prevalence statistics from several Asian research, over 60% of Asian countries (Suzuki and Yamamoto, 2009) are affected by *Campylobacter* contamination. This value is merely approximate data for retail chicken meat with by-products, and the on-farm frequency of *Campylobacter* spp across European chicken flocks ranges from 18% to 90% (European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) et al., 2019). The rates of infection in several Chinese provinces and cities, such as *C. jejuni* contamination in Baoding (56.0%) and chicken fecal swab contamination in Nanjing (26.8%), have been reported (Wang et al., 2013). A total of 977 strains of *C. jejuni* were identified from chickens during the testing for *Campylobacter* in the intestinal contents and excreta of chickens from farms and slaughterhouses in 5 Chinese provinces; the contaminated samples accounted for 18.1% of samples obtained from flocks (Wang et al., 2016).

In this work, the positive rate of *C. jejuni* observed in broiler farms in central Shanxi Province, China was 10.8%, which was lower than that observed in other parts of China and some other countries. The investigation of the source of infection in farms aims to provide a basic reference data for *C. jejuni* infections, including human and animal infections. Chicken carcasses are easily contaminated during slaughter, thus causing human infection. The statistical analysis of the results of this experiment showed differences in the positivity rates of cloacal swabs between broiler farms in different regions. A significant difference ($P < 0.05$) was found between the prevalence of positive rates in Lvliang (3.7%) and Jinzhong farms (18.0%). A significant difference ($P < 0.05$) was also found between the prevalence of positive rates in Yangquan (2.8%) and Jinzhong farms (2.8%), with the prevalence being highest in Jinzhong farms. This difference could be due to the closed environment of broiler farms in the Jinzhong area, as well as intensive cage breeding and other factors. The different collection sites and samples collected in different countries and regions, as well as the influence of factors, such as management style and geographical health environment, caused the findings to vary, resulting in different prevalence rates. Controlling the carriage rate of *C. jejuni* in chickens, the main host of *C. jejuni*, is important for the control of campylobacteriosis in the population.

C. jejuni is becoming increasingly resistant to clinically important antibiotics, and drug-resistant strains can be transmitted to humans through the food chain; this situation constitutes a major public health problem (Wangroongsarb et al., 2021). In this study, chicken *C. jejuni* isolates had high resistance to quinolones and β-lactams with rates similar to those reported in previous studies (approximately 80%) (Luangtongkum et al., 2009). Meanwhile, most strains were still sensitive to gentamicin and erythromycin. The findings of this test were consistent with those of Wieczorek et al., 2017, who reported that 92.5% of *C. jejuni* isolates from Polish flocks were resistant to quinolones but not to erythromycin. Rivera et al. (2011) also discovered that *C. jejuni* had minimal resistance to erythromycin and gentamicin. The multiple drug resistance rate of 88.6% determined through the drug sensitivity test in this work was not significantly different from the findings reported for other areas in China as follows: 71.7% in Shanghai, 90.0% in Shandong Province, and 91.3% in Sichuan Province (Chen et al., 2010; Ma et al., 2014; Han et al., 2016) and was rather lower than that abroad. Bacteria were resistant to 5 or more antibiotics, including norfloxacin and ampicillin. Therefore, new antibiotics or combination medications are needed to minimize *C. jejuni* infections and transmission in hens.

In this study, MLST typing was used for the first time to investigate the genotypes of chicken-derived *C. jejuni* from farms in some areas of Shanxi. The genetic diversity of these strains was analyzed and revealed. The sequence types of all isolated strains revealed that in addition to chickens, humans, ducks, and dogs were the sources of *C. jejuni* and were indicative of the diversity of the transmission of *C. jejuni* infection. Furthermore, the similarity of the distribution of the same sequence type between chicken and human isolates suggested that at least some cases of *C. jejuni* disease were linked to the consumption of contaminated chicken, meat products, or other food cross-contaminated with *C. jejuni*, with direct contact with *C. jejuni*-infected chickens being another possibility. Genetic evolution analysis revealed that strains with the same sequence type originated from the same area, isolates from different regions had unique sequence types, and a few sequence types were cross-distributed in broiler farms in different regions. Moreover, some sequence types were genetically similar to sequence types from other nations or sources in the database. The PubMLST database of *C. jejuni* states that the most isolated clonal complex of *C. jejuni* globally is ST-21, followed by ST-828 and ST-45. In a study

on *C. jejuni* sequence types in northern China, the ST-21 clonal complex was shown to be the most prevalent among human and chicken isolates, followed by the ST-353 complex (Ma et al., 2017). In the present work, the most common clonal complex was ST-354 (14.3%), followed by ST-21 and ST-828. These results were somewhat different from the global prevalence and previous findings.

We also investigated the prevalence of 6 virulence factor genes in the DNA of the tested *C. jejuni* isolates. The colonization and pathogenicity of *Campylobacter* are linked to virulence factors, such as adhesion, invasive ability, and toxin generation factors. The capacity of the *cad*F gene to promote bacterial adhesion by binding to host fibronectin through attachment to host epithelial cells is a vital step in the manifestation of pathogenicity in *Campylobacter*, and this ability aids the colonization and invasion of the host organism (Krause-Gruszczynska et al., 2007). Alterations in other genes related to flagellar motility (*fla*A), chemotaxis-assisted invasion (*che*Y), and heat shock protein (*dna*J) would affect colonization in the intestine (Konkel et al., 1998). The extracellular toxin gene *cdt*B is regarded as the principal virulence gene of Gram-negative bacteria. Our study found that the prevalence of 5 virulence genes was high and that *cad*F was present in all strains. The high prevalence rates of *cad*F and *cdt*B in this work were similar to those in many investigations that observed similar conditions in practically all *Campylobacter* isolates (Abu-Madi et al., 2016). However, the detection rate of *cia*B, which is associated with invasion, in this work was only half of that in other studies (Datta et al., 2003; Abu-Madi et al., 2016). This situation may lead to a reduction in the invasiveness of the strain.

ACKNOWLEDGMENTS

This study was funded by the grants from the National Natural Science Foundation of China (Grant No. 31602029), the Basic Research Project of Shanxi Province (Grant No. 202103021224165), and the Major Research Project of Jinzhong City (Grant No. Y212010).

DISCLOSURES

Hui-lin Yang carried out most of the experiments, wrote the manuscript, and should be considered as primary author. Rui Bai critically revised the manuscript and the experiment design. Yong-bin Li, Yu Zhang, Bowen Dong, Bu-ting Duan, Lu-lu Guo, Ting-yang Wang, Xiao-ling Lv, Xiao-zhen Cui, and Ming-Xue Zheng helped with the experiment. All the authors read and approved the final version of the manuscript.

REFERENCES

Abu-Madi, M., J. M. Behnke, A. Sharma, R. Bearden, and N. Al-Banna. 2016. Prevalence of virulence/stress genes in *Campylobacter jejuni* from chicken meat sold in Qatari retail outlets. PLoS One 11:e0156938.

Cerf, O., B. Carpentier, and P. Sanders. 2010. Tests for determining in-use concentrations of antibiotics and disinfectants are based on entirely different concepts: "resistance" has different meanings. Int. J. Food Microbiol. 136:247–254.

Chen, X., G. W. Naren, C. M. Wu, Y. Wang, L. Dai, L. N. Xia, P. J. Luo, Q. Zhang, and J. Z. Shen. 2010. Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Vet. Microbiol.* 144:133–139.

Colles, F. M., R. J. Cain, T. Nickson, A. L. Smith, S. J. Roberts, M. C. Maiden, D. Lunn, and M. S. Dawkins. 2016. Monitoring chicken flock behaviour provides early warning of infection by human pathogen *Campylobacter*. Proc. Biol. Sci. 283:20152323.

Colles, F. M., and M. C. J. Maiden. 2012. *Campylobacter* sequence typing databases: applications and future prospects. Microbiology (Reading) 158(Pt 11):2695–2709.

Datta, S., H. Niwa, and K. Itoh. 2003. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. J. Med. Microbiol. 52(Pt 4):345–348.

Dingle, K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J. Bootsma, R. J. Willems, R. Urwin, and M. C. Maiden. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J Clin. Microbiol. 39:14–23.

EFSA Panel on Biological Hazards. 2009. Joint opinion on antimicrobial resistance (AMR) focused on zoonotic infections. EFSA J. 7:1372.

Epps, S. V., R. B. Harvey, M. E. Hume, T. D. Phillips, R. C. Anderson, and D. J. Nisbet. 2013. Foodborne *Campylobacter*: infections, metabolism, pathogenesis and reservoirs. Int. J. Environ. Res. Public Health 10:6292–6304.

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). 2019. The European Union one health 2018 Zoonoses report. EFSA J. 17:e05926.

Fothergill, A. W. 2012. Antifungal susceptibility testing: Clinical Laboratory and Standards Institute (CLSI) methods. Pages 65−74 in Interactions of Yeasts, Moulds, and Antifungal Agents. Springer, Berlin.

Goualie, B. G., E. E. Akpa, S. E. Kakou-N'Gazoa, H. G. Ouattara, S. L. Niamke, and M. Dosso. 2019. Antimicrobial resistance and virulence associated genes in *Campylobacter jejuni* isolated from chicken in Cote d'Ivoire. J. Infect. Dev. Ctries. 13:671–677.

Han, X. F., D. M. Zhu, H. M. Lai, H. Zeng, K. Zhou, L. K. Zou, C. M. Wu, G. Q. Han, and S. L. Liu. 2016. Prevalence, antimicrobial resistance profiling and genetic diversity of *Campylobacter jejuni* and *Campylobacter coli* isolated from broilers at slaughter in China. *Food Control* 69:160–170.

Hickey, T. E., A. L. McVeigh, D. A. Scott, R. E. Michielutti, A. Bixby, S. A. Carroll, A. L. Bourgeois, and P. Guerry. 2000. *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. *Infect. Immun.* 68:6535–6541.

Kaakoush, N. O., N. Castano-Rodriguez, H. M. Mitchell, and S. M. Man. 2015. Global epidemiology of *Campylobacter* infection. Clin. Microbiol. Rev. 28:687–720.

Konkel, M. E., S. A. Gray, B. J. Kim, S. G. Garvis, and J. Yoon. 1999. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter* coli based on the cadF virulence gene and its product. J. Clin. Microbiol. 37:510–517.

Konkel, M. E., B. J. Kim, J. D. Klena, C. R. Young, and R. Ziprin. 1998. Characterization of the thermal stress response of *Campylobacter jejuni*. Infect. Immun. 66:3666–3672.

Krause-Gruszczynska, M., M. Rohde, R. Hartig, H. Genth, G. Schmidt, T. Keo, W. Konig, W. G. Miller, M. E. Konkel, and S. Backert. 2007. Role of the small rho gtpases rac1 and cdc42 in host cell invasion of *Campylobacter jejuni*. *Cell Microbiol.* 9:2431–2444.

Luangtongkum, T., B. Jeon, J. Han, P. Plummer, C. M. Logue, and Q. Zhang. 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol. 4:189–200.

Ma, H., Y. Su, L. Ma, L. Ma, P. Li, X. Du, G. Golz, S. Wang, and X. Lu. 2017. Prevalence and characterization of *Campylobacter jejuni* isolated from retail chicken in Tianjin, China. *J. Food Prot.* 80:1032–1040.

Ma, L., Y. Wang, J. Shen, Q. Zhang, and C. Wu. 2014. Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. Int. J. Food Microbiol. 181:77–84.

Maesaar, M., T. Kramarenko, K. Meremae, J. Sogel, M. Lillenberg, L. Hakkinen, M. Ivanova, K. Kovalenko, A. Horman, M. L. Hanninen, and M. Roasto. 2016. Antimicrobial resistance profiles of *Campylobacter* spp. Isolated from broiler chicken meat of Estonian, Latvian and Lithuanian origin at Estonian retail level and from patients with severe enteric infections in Estonia. *Zoonoses Public Health* 63:89–96.

Man, S. M. 2011. The clinical importance of emerging *Campylobacter* species. Nat. Rev. Gastroenterol. Hepatol. 8:669–685.

Marotta, F., G. Garofolo, G. Di Donato, G. Aprea, I. Platone, S. Cianciavicchia, A. Alessiani, and E. Di Giannatale. 2015. Population diversity of *Campylobacter jejuni* in poultry and its dynamic of contamination in chicken meat. Biomed. Res. Int. 859845.

Nguyen, T. N., H. Hotzel, H. El-Adawy, H. T. Tran, M. T. Le, H. Tomaso, H. Neubauer, and H. M. Hafez. 2016. Genotyping and antibiotic resistance of thermophilic *Campylobacter* isolated from chicken and pig meat in Vietnam. *Gut. Pathog.* 8:19.

Rivera, F. N., B. R. Bustos, H. S. Montenegro, M. M. Sandoval, N. J. Castillo, J. H. Fernandez, R. M. Maturana, R. L. Delgado, S. A. Contreras, N. D. Chavez, and L. I. Quevedo. 2011. Genotyping and antibacterial resistance of *Campylobacter* spp strains isolated in children and in free range poultry. *Rev. Chilena. Infectol.* 28:555–562.

Suzuki, H., and S. Yamamoto. 2009. *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. J. Vet. Med. Sci. 71:255–261.

Tangkham, W., M. Janes, and F. LeMieux. 2016. Prevalence and distribution of *Campylobacter jejuni* in small-scale broiler operations. J. Food Prot. 79:75–81.

Thrusfield, M. 2018. Veterinary Epidemiology. John Wiley & Sons, America.

Wang, Y., Y. Dong, F. Deng, D. Liu, H. Yao, Q. Zhang, J. Shen, Z. Liu, Y. Gao, C. Wu, and Z. Shen. 2016. Species shift and multidrug resistance of *Campylobacter* from chicken and swine, China, 2008-14. J. Antimicrob. Chemother. 71:666–669.

Wang, J., Y. C. Guo, and N. Li. 2013. Prevalence and risk assessment of *Campylobacter jejuni* in chicken in china. Biomed. Environ. Sci. 26:243–248.

Wangroongsarb, P., N. Cheunban, C. Jittaprasatsin, T. Kamthalang, N. Saipradit, P. Chaichana, C. Pulsrikarn, and S. Parnmen. 2021. Prevalence and antimicrobial susceptibility of Campylobacter isolated from retail chickens in Thailand. Int J Food Microbiol 339:109017.

Wieczorek, K., E. Denis, B. Lachtara, and J. Osek. 2017. Distribution of *Campylobacter jejuni* multilocus sequence types isolated from chickens in Poland. Poult. Sci. 96:703–709.

Zendehbad, B., A. A. Arian, and A. Alipour. 2013. Identification and antimicrobial resistance of *Campylobacter* species isolated from poultry meat in Khorasan Province, Iran. Food Control 32:724–727.